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## **Final Report**

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**Effects of Space Flight on Rodent Tissues**

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## **Abstract**

As the inevitable expression of mankind's search for knowledge continues into space, the potential acute and long-term effects of space flight on human health must be fully appreciated. Despite its critical role relatively little is known regarding the effects of the space environment on the ocular system. Our proposed studies were aimed at determining whether or not space flight causes discernible disruption of the genomic integrity, cell kinetics, cytoarchitecture and other cytological parameters in the eye

Because of its defined and singular biology our main focus was on the lens and possible changes associated with its primary pathology, cataract. We also hoped to explore the possible effect of space flight on the preferred orientation of dividing cells in the perilimbal region of conjunctiva and cornea.

## **Objectives**

The principle goals were to define and characterize the effect of space flight on the genomic integrity, cell kinetics, cytoarchitecture, cell apoptosis, and cataract-related cytopathological indicators. In addition the possibility of an influence on the preferred orientation of dividing cells in conjunctival epithelium will also be studied.

Among the rationales for such studies are: (1) any changes which may occur in the lens are not likely to be unique to that tissue and therefore may be indicators of more pervasive effects of microgravity and/or radiation on integrated tissues in general, (2) the preferred orientation of dividing cells in conjunctival epithelium - a process theorized to be critical for maintaining the structure and function of cornea and conjunctiva - is mechanistically dependent on spatial and temporal controls and therefore may be highly sensitive to changes in gravitational field.

## **Specific aims**

Our specific aims, therefore, were:

1. To observe space flight effect on integrated biological systems by assessing cytopathological indicators in lens epithelium. These include the analysis of mitotic transit time and activity, cell death and genomic integrity in the lens epithelium.

2. To determine the possibility of a cataractotoxic contribution by microgravity and/or radiation during space flight by analyzing cytological indicators in the lens epithelium known to presage the development of cataract. These include Meridional Row (MR) disorganization, Micronuclei frequency and cell cycle effects.
3. To determine the space flight effect on the accuracy preferred orientation of dividing cells in conjunctival epithelium.

## **Rationale**

The concerns of our laboratory have been on the effects of space related radiation on three primary tissues of the eye; the cornea/conjunctiva, lens and retina (Worgul et al., 1993, Brenner et al., 1991, Krebs et al., 1990, Worgul et al., 1989, Worgul et al., 1989, Worgul et al., 1989, Tao et al., 1993, Tao et al., 1993, Vazquez and Worgul, 1993). These tissues have been studied because of their amenability to ready study, their long histories as indicators of potential damage from exogenous physical and chemical agents, and the importance of their integrity to continued ocular function. In addition, each of these tissues has qualities which allows it to serve as a paradigm for related tissue systems elsewhere in the body.

Our studies to date have been directed on the potential damage from exposure to galactic cosmic radiation which might be incurred during extended deep space flight. While our ground-based studies are leading us to an appreciation on the effects of accelerated heavy ions on these tissues, the potential influence of microgravity is still a major issue of concern. This is particularly true, inasmuch as in other systems there has been some suggestion of a possible synergism (Reitz et al., 1989, Planel, et al., 1989). To properly address these questions we must await dedicated rodent experiments aboard extended duration missions such as the EDO or Freedom. However, in the meantime, it is appropriate that we begin to obtain baseline data on the influence of microgravity during even short duration flights. At a minimum the scientific experience gained from such studies will allow us to appropriately bracket the nature of the controls which should be incorporated into future flight validation studies. We will also gain procedural experience in the logistics of tissue handling and develop strategies to deal with NASA constraints at the level of implementation of our requirements.

As outlined in our original proposal NASA's "Biological Flight Experiments Tissue Sharing Program" enabled us to obtain tissues from missions STS-48 (PARE. 01) and STS-54 (PARE. 02). While STS-48's unexpected West Coast landing resulted in poor preservation of the eye tissues and consequently, compromised results, analysis of lenses from STS-54 produced some provocative results. There was a statistically significant increase in the number of mitotic aberrations. When the mitotic activity was determined a significant alteration in transit time was also noted.

Other parameters tended to show an effect of flight versus non-flight conditions, although the small sample size militated against demonstrating statistical significance. The MR disorganization in flight eyes is more severe than the controls. This is very important because MR organization is critical in maintaining the subsequent cellular differentiation and fiber formation in proper order. A failure in the process results in cataractogenesis. Of equal moments was the tendency toward a higher MN frequency. Micronucleation is an indicator of genotoxicity. In the lens an increase in MN frequency presages the expression of aberrant differentiation.

Finally the number of apoptotic cells (diagnosed by their fragmented nuclei - FN), in flight lenses was obviously higher than controls. Again however the available sample was too low to demonstrate significance. Believed to be an important process involved in disorders of other biological systems, such as carcinogenesis, its effect on lenticular integrity is unknown.

### **Results of SLS-2 Tissue Analysis:**

We had hoped to resolve the statistical shortcomings by analyzing the tissues from the SLS-2 mission. The ERERL received the eyes of 40 animals associated with the SLS-2 mission which flew on October 18, 1993. Dr Feng Tao who was experienced in ocular cytopathology was assigned to process the tissues. Despite intensive and meticulous care in their handling it was clear that the tissues were not usable. The fixation was very poor and the tissues were degraded. We suspect that the problem lay in a failure to create a slit in the globe (as described in our instructions – page 6, step 6) to facilitate entry of the fixative. After extensive effort none of the eyes provided sufficiently intact tissue to allow reliable analysis and we could therefore conclude nothing from the experiment.

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